Pharmacokinetic and Other Factors Related to Mutagenicity Testing: Quantitative Analysis of the Testing Procedures

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I would like to focus attention first on three major and immediate problem areas inherent in any testing system and then to discuss to a limited extent a major problem, generally not discussed, involved in the elucidation of the meaningfulness of any test system for human health and welfare.

The three problem areas I wish to discuss first are: (1) the toxic agent—the suspect compound; (2) the target—the tissue likely to develop the toxic response; and (3) the time factors involved in test systems.

There are three questions that must be asked regarding the toxic agent. What chemical is the toxic agent? How much of that agent is present? How long is that agent present? These questions must be asked whether one is doing an experiment on *Neurospora*, on human cells *in vitro*, on laboratory animals, individually on man, or on the human population. In more classical terminology, the question of the identity of the agent refers to studies on metabolic alterations of the compounds by cellular activity. The question of amount or concentration of agent is the function of not only the metabolism but also the distribution and the environmental exposure and absorption rates. How long the susceptible cells are exposed to the toxic agent is a function of environmental exposure, distribution, metabolism excretion, and absorption, and these are all problems which might best be described as the pharmacological disposition of the toxic agent.

That the human body or the bacterial cell has the capability metabolically to alter foreign organic compounds is well known. It is, however, becoming increasingly apparent that in the hazardous process of attempting to extrapolate data for laboratory test systems to man, the major impediment is the different metabolic patterns in the various species of test organisms and man. These differences may be both qualitative in that different chemical metabolites may be produced and quantitative in that different rates of production may occur. The ability of the hepatic microsomal mixed function oxidase system to alter a great variety of foreign organic compounds is well known indeed, and I will not discuss it further in any great detail (*1–3*). It may be worthwhile to point out certain generalizations which apply to this system in mammals. First, lipid-soluble compounds are rendered less lipid-soluble and more

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polar by this system. Second, small animals tend to have a more rapid metabolism of foreign compounds than large animals. Third, herbivorous animals tend to metabolize compounds more actively than do carnivorous animals.

It is important to recognize that metabolic alteration of administered compounds may occur in the gut even before absorption has occurred. This may vary between species or within species; variation may even occur in the same individual at different times and on different diets. We are all familiar with the fact that sodium cyclamate becomes the more toxic cyclohexylamine by action of bacteria in the gastrointestinal tract. It is also becoming increasingly apparent that there are sites of metabolism in the vertebrate body beyond the liver. The lung, the testes, the kidney, and other organs may play important roles in the metabolism of the administered compound.

The age and the sex of the animals can be important. These and other physiological factors can influence the metabolism of foreign compounds. Factors external to the animal may also be important. The ability of one therapeutic drug to affect the metabolism drastically and therefore alter the toxicity or activity of a second therapeutic drug is now well known. It seems likely that this will be demonstrated for environmental chemicals. Thus, in the design of meaningful and effective test systems and in the extrapolation of data from these test systems to man, one must constantly ask whether the agent being tested is the original compound or one or more metabolites.

The second major concern is how much of the compound—be it the original agent or a more toxic metabolite—is in contact with the target tissue. Here it is necessary to look at what is described as the pharmacokinetics of a compound. This includes a variety of factors. The rate of absorption through the skin, the lungs, or the gastrointestinal tract is important. It must be recognized that the hydrogen ion concentration of the stomach can affect the rate and extent of absorption of partially ionized compounds. For slowly absorbed compounds, the gastrointestinal tract surface to volume ratio and transit time can be of considerable importance, and these vary among species. The route of administration in the experimental situation may be important. There is good evidence that after an intraperitoneal injection almost all of the compound flows through the hepatic system and therefore is susceptible to metabolism before it reaches the rest of the body. With a subcutaneous or intravenous injection this is not true, and the first passage of the compound to the other tissues of the body is in its unaltered state.

The question of how long the toxic agent stays in contact with susceptible tissue is also important. There may be delays in absorption or penetration that lead to prolonged, low tissue concentrations. This, of course, is a function not only of the absorption and metabolism, but also of two other major factors. The first is the excretory rate. Primary routes of excretion are renal and biliary. It is clear that the small mammals excrete compounds by the renal route much more rapidly than do large mammals (*4*). Biliary excretion is a complex active process which has not been well studied in a comparative way (*5*). One must take into consideration not only the immediate biliary excretion but also the potential reabsorption of the compound from the gastrointestinal tract after it has been excreted.

The distribution rate of the compound after absorption throughout the body is of major importance. This is well illustrated by the following facts: the cardiac output of the mouse is such that the blood volume is circulated 20 times per minute, while the cardiac output of man is such that the blood volume is circulated once per minute. Thus, the rate of distribution and mixing and delivery to the tissues of a compound within the body of a small mammal is very much more rapid than that in the body of a large mammal. Dedrick and co-workers have developed these principles in an important series of studies (*6*).

The concept which follows from these comparative studies on metabolic alteration,

excretory rate, and distribution is consistent with the notion that small mammals dispose of (i.e., distribute, metabolize, and excrete) foreign organic compounds at a more rapid rate than do large mammals. This overall observation is supported by older empirical data on the relative toxicity of anticancer drugs in laboratory mice, rats, hamsters, dogs, monkeys, and in man. The lethal toxicity, or the maximum tolerated dose in man, was close to identical to the dose in the experimental animals if adjusted to a unit dose per meter surface area or unit dose per weight to the two-thirds power. This suggests that on a milligram per kilo basis the mouse is in fact 10 to 12 times more resistant to an average compound than is man (*7*).

These considerations are important first in the *in vitro* experiments where proper estimates of concentration and duration must be made, also in experiments with laboratory animals, and finally in man, if one is actually to predict toxicity to man.

The last problem is one of the tissue target itself. There are really three aspects to this. The first concerns the problem of barriers between the general circulation and the susceptible cell or area within the cell. The blood–brain barrier is well known; the blood–testicular barrier has been described by Dixon (*8*); other barriers may well exist within the body. Second is the question of the innate susceptibility of the cell. For example, Flamm describes the differences in repair mechanisms between bacteria in rodents and man (*9*), which can influence the apparent innate susceptibility of the tissue target. Finally, I think it is important to consider the number—the quantity—of the susceptible cells in the test systems relative to those in man. This is particularly important when searching for a rare event. If an agent induces a mutation rate of 10^{-6} but there are only 10^{4} cells available, it would be unlikely that a mutation would be detected. These considerations I think deserve much more attention than they have had in the past.

The last factor related to the toxic agent that I will discuss is the time—by this I mean the duration of the exposure. Chemical mutagenesis tests as currently designed consist of a brief exposure to the test animal or the test organism of the suspect compound. Very often, however, the real life situation is one of either a long-term, low-dose exposure or repeated small or moderate exposures. To what extent these are comparable I think is not known.

Another aspect of time is the ability of the human to accumulate compounds which are slowly excreted and/or metabolized over decades of exposure. For instance, in the mouse 2 ppm of DDT in the diet will yield, after about 1½ yr of exposure, a concentration in the fat of 5 to 6 ppm of DDT and its metabolites (*10*). This is about the same concentration present in the fat of the average U.S. citizen after decades of exposure at an exposure rate of about 0.015 to 0.04 ppm in his diet (*11*).

In the process of comparing *in vitro* studies or laboratory animal studies with man, it is necessary to consider all of the factors mentioned above: those related to the toxic agent, those related to the target tissue, and those related to time. We must be sure that in all cases we are considering the same chemical at the same concentration for the same duration in the test system as is the case in the human situation. Unfortunately, even with proper and extensive consideration of these factors, important problems remain. It would be inadequate to compare what I call the "median" or average mouse to the median or average man, or the median *Drosophila* to the median man, or the median *Tradescantia* to the median man, or the median *Neurospora* to the median man. Our aim is to protect from the toxic effects of environmental chemicals more than just the median or the average man; it is to protect a very large fraction of the human population. It is beyond the realm of this discussion to consider what that proper fraction should be. To put it simply, to estimate safe levels for human exposure we must be concerned about human variability, about exposure variability, about environmental variability, and about synergistic variability. Each one of these factors

can render what is apparently a safe concentration or exposure in an ideal situation into an exposure that can cause toxicity. It is known that for certain lipid-soluble therapeutic drugs the steady-state plasma concentration in man can vary by a factor of 30-fold between individual patients when the same dose is given to each patient. It is known that for certain compounds the half-life plasma in a small population of patients can vary 10-fold (*12*). Thus, knowledge of the average or median rate of drug disposition for the average man may not give much information as to the drug disposition rate of that 5% or 1% or 0.1% of the population that is either "slow" or "fast."

We know there can be a very great variability in the actual exposure of environmental agents to man. We know that environmental effects themselves can affect metabolism, distribution, and excretion, as well as response, of foreign compounds, and these must be taken into consideration. Lastly, and perhaps most importantly, we must be particularly aware of the possibility of synergistic toxic interactions. It is well demonstrated for therapeutic drugs that one agent can drastically increase the toxicity of a second therapeutic agent. While this has not been well demonstrated for environmental agents, it seems very likely that it also occurs. Studies on synergistic toxicity might be ideal for *in vitro* methodology in which large numbers of compounds could be run alone and in combination very easily. It seems to me that, as we consider our test systems and the population that we want to protect, we must be aware of the toxic agent, the target cell, the time factor, and the variability factors. We must try very hard to achieve the same concentrations of the same chemical for the same time in the same tissues in the test systems as we do in man. When we have done that we have not yet succeeded, because we almost must anticipate the variability intrinsic in man and his complex way of life.

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